L5 ANSWER 18 OF 33 MEDLINE
ACCESSION NUMBER: 95347605 MEDLINE
DOCUMENT NUMBER: 95347605 PubMed ID: 7622057
TITLE: Microbial secretion of biologically active human transforming growth factor alpha fused to the Streptomyces protease inhibitor.
AUTHOR: Taguchi S; Misawa S; Yoshida Y; Momose H
CORPORATE SOURCE: Department of Biological Science and Technology, Science University of Tokyo, Chiba, Japan.
SOURCE: GENE, (1995 Jul 4) 159 (2) 239-43.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950911
Last Updated on STN: 19950911
Entered Medline: 19950831

AB A secretory production system for the active form of transforming growth factor alpha (TGF alpha) was established in Streptomyces lividans using a gene encoding the secretory protease inhibitor, Streptomyces subtilisin inhibitor (SSI). It was demonstrated that deletion of one of the putative dual ssi terminators is effective to extracellularly produce a **heterologous** polypeptide in a fused form. The recombinant fusion protein, SSI::TGF alpha, was purified to homogeneity by a combination of hydrophobic chromatography and reverse-phase high-performance liquid chromatography (RP-HPLC). It was noteworthy that the SSI::TGF alpha **hybrid protein** exhibited bifunctional activity: the TGF alpha activity for cell growth promotion and the inhibitory activity of SSI. Taken together with the results of analytical gel filtration, these findings strongly indicate that each moiety in the fusion protein correctly folds and the whole hybrid molecule exists in a dimeric form, which results in its bifunctional activity.

L5 ANSWER 17 OF 33 MEDLINE
ACCESSION NUMBER: 96004457 MEDLINE
DOCUMENT NUMBER: 96004457 PubMed ID: 7551026
TITLE: Recombinant BCG expressing the leishmania surface antigen Gp63 induces protective immunity against Leishmania major infection in BALB/c mice.
AUTHOR: Abdelhak S; Louzir H; Timm J; Blel L; Benlasfar Z; Lagranderie M; Gheorghiu M; Dellagi K; Gicquel B
CORPORATE SOURCE: Unite de Genetique Mycobacterienne (CNRS URA 1300), Institut Pasteur de Paris, France.
SOURCE: MICROBIOLOGY, (1995 Jul) 141 (Pt 7) 1585-92.
Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 20000303
Entered Medline: 19951120

AB We have cloned and expressed the gp63 gene of Leishmania major in BCG to develop a recombinant vaccine against zoonotic cutaneous leishmaniasis. Two different expression systems were investigated. The first system consists of pAN, a Mycobacterium paratuberculosis promoter, which drives expression of ORF2, an open reading frame in IS900. This system allows the production of **heterologous** polypeptides as hybrids with the ORF2 gene product. The second expression system relies on the production of antigenic fragments as fusion proteins with the N-terminal region of Mycobacterium fortuitum beta-lactamase. Both constructs resulted in the production of Gp63 in BCG. The ability of the two recombinant BCG strains to induce protective immunity against a challenge with L. major amastigotes was evaluated after vaccination of susceptible (BALB/c), and resistant (C57BL/6) mice. Recombinant BCG producing Gp63 as a **hybrid protein** with the N-terminal region of the beta-lactamase elicited significant protection against a challenge with L. major in BALB/c-immunized mice.

L5 ANSWER 9 OF 33 MEDLINE
ACCESSION NUMBER: 97158650 MEDLINE
DOCUMENT NUMBER: 97158650 PubMed ID: 9006010
TITLE: Linker mutagenesis of the Caulobacter crescentus S-layer protein: toward a definition of an N-terminal anchoring region and a C-terminal secretion signal and the potential for **heterologous** protein secretion.
AUTHOR: Bingle W H; Nomellini J F; Smit J
CORPORATE SOURCE: Department of Microbiology and Immunology, The University of British Columbia, Vancouver, Canada.
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Feb) 179 (3) 601-11.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970313
Last Updated on STN: 19970313
Entered Medline: 19970228

AB Linker insertion mutagenesis was used to modify the paracrystalline surface layer (S-layer) protein (RsaA) of the gram-negative bacterium Caulobacter crescentus. Eleven unique BamHI linker insertions in the cloned rsaA gene were identified; at the protein level, these linker insertions introduced 4 to 6 amino acids at positions ranging from the extreme N terminus to the extreme C terminus of the 1,026-amino-acid RsaA protein. All linker-peptide insertions in the RsaA N terminus caused the secreted protein to be shed into the growth medium, suggesting that the RsaA N terminus is involved in cell surface anchoring. One linker-peptide insertion in the RsaA C terminus (amino acid 784) had no effect on S-layer biogenesis, while another (amino acid 907) disrupted secretion of the protein, suggesting that RsaA possesses a secretion signal lying C terminal to amino acid 784, near or including amino acid 907. Unlike extreme N- or C-terminal linker-peptide insertions, those more centrally located in the RsaA primary sequence had no apparent effect on S-layer biogenesis. By using a newly introduced linker-encoded restriction site, a 3' fragment of the rsaA gene encoding the last 242 C-terminal amino acids of the S-layer protein was expressed in C. crescentus from **heterologous** Escherichia coli lacZ transcription and translation initiation information. This C-terminal portion of RsaA was secreted into the growth medium, confirming the presence of a C-terminal secretion signal. The use of the RsaA C terminus for the secretion of **heterologous** proteins in C. crescentus was explored by fusing 109 amino acids of an envelope glycoprotein from infectious hematopoietic necrosis virus, a pathogen of salmonid fish, to the last 242 amino acids of the RsaA C terminus. The resulting **hybrid protein** was successfully secreted into the growth medium and accounted for 10% of total protein in a stationary-phase culture. Based on these results and features of the RsaA primary sequence, we propose that the C. crescentus S-layer protein is secreted by a type I secretion system, relying on a stable C-terminal secretion signal in a manner analogous to E. coli alpha-hemolysin, the first example of an S-layer protein secreted by such

L1 ANSWER 47 OF 139 MEDLINE
ACCESSION NUMBER: 1999421688 MEDLINE
DOCUMENT NUMBER: 99421688 PubMed ID: 10491129
TITLE: Functional complementation analysis of **yeast** bc1 mutants. A study of the mitochondrial import of **heterologous** and **hybrid** proteins.
AUTHOR: van Wilpe S; Boumans H; Lobo-Hajdu G; Grivell L A; Berden J A
CORPORATE SOURCE: Section for Molecular biology, Department of Molcular Cell Biology, BioCentrum, University of Amsterdam, The Netherlands.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 Sep) 264 (3) 825-32.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991014

AB Previous complementation studies with **yeast** bc1 mutants, defective in subunit VII or VIII, using **heterologous** and **hybrid** subunits, suggested that the requirement for import into mitochondria might significantly restrict the scope of this test for compatible proteins. Prediction algorithms indicate that the N-terminal domain of subunit VII contains all known characteristics of a mitochondrial targeting signal, whereas in subunit VIII such a signal is absent from the N-terminal domain, but possibly present in an internal region of the protein. Despite the fact that the characteristics of a mitochondrial import signal are found in the N-terminus of all known subunit-VII orthologues, in vitro import experiments show that the protein of human origin is not imported into **yeast** mitochondria. In vitro import can be restored, however, by replacement of the N-terminal part of the human protein by the N-terminus of the Saccharomyces cerevisiae orthologue, indicating a requirement for species-specific elements. Similar experiments were performed with subunit VIII and orthologues thereof, including a **hybrid** protein in which the N-terminus of the bovine heart orthologue was replaced by that of S. cerevisiae. The ability of **yeast** mitochondria to import this **hybrid** protein, in contrast with the bovine subunit-VIII orthologue itself, indicates that for subunit VIII also the N-terminus, in contradiction of theoretical predictions, contributes to the targeting signal, most likely via species-specific elements. Our findings expose the limitations of the currently available criteria for prediction of the presence and location of a mitochondrial targeting sequence and highlight the necessity of performing separate import studies for interpreting complementation studies as long as the species-specific characteristics of

L3 ANSWER 58 OF 64 MEDLINE
ACCESSION NUMBER: 88015615 MEDLINE
DOCUMENT NUMBER: 88015615 PubMed ID: 2821510
TITLE: The production of **hybrid** Ty:IFN virus-like particles in **yeast**.
AUTHOR: Malim M H; Adams S E; Gull K; Kingsman A J; Kingsman S M
CORPORATE SOURCE: Department of Biochemistry, University of Oxford, UK.
SOURCE: NUCLEIC ACIDS RESEARCH, (1987 Sep 25) 15 (18) 7571-80. Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198711
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19990129
Entered Medline: 19871118

AB The **yeast** retrotransposon Ty encodes proteins that assemble into virus-like particles (Ty-VLPs) which can be readily purified. We have recently shown that expression of the p1 protein encoded by the TYA gene of Ty is sufficient for particle formation. In this paper we show that when a **heterologous** coding sequence, human interferon-alpha 2 (IFN), is fused in frame to the TYA gene, the resulting p1-IFN fusion protein is still assembled into VLPs. These Ty:IFN-VLPs can be easily purified to near homogeneity and furthermore, they induce an antibody response to interferon when they are injected into rabbits. Therefore, these data show that **hybrid** Ty-VLPs can be used as a convenient

L3 ANSWER 56 OF 64 MEDLINE
ACCESSION NUMBER: 88185830 MEDLINE
DOCUMENT NUMBER: 88185830 PubMed ID: 3328732
TITLE: High level expression of proinsulin in the **yeast**, Saccharomyces cerevisiae.
AUTHOR: Cousens L S; Shuster J R; Gallegos C; Ku L L; Stempien M M; Urdea M S; Sanchez-Pescador R; Taylor A; Tekamp-Olson P
CORPORATE SOURCE: Chiron Corporation, Emeryville, CA 94608.
SOURCE: GENE, (1987) 61 (3) 265-75.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19880524

AB Human proinsulin (PI) has been expressed to a high level (100 mg/liter) as a human superoxide dismutase-PI fusion protein in the **yeast**, Saccharomyces cerevisiae. At the junction of the two proteins is a methionine residue, allowing PI to be released from the fusion by reaction with cyanogen bromide. The fusion is expressed using a regulated, **hybrid** promoter containing the regulatory region of the alcohol dehydrogenase II promoter and the 3' end of a glyceraldehyde-3-phosphate dehydrogenase promoter, allowing the recombinant **yeast** cells to be stably maintained. Production of the fusion protein is induced by growth in medium lacking a fermentable carbon source. The **heterologous** fusion protein is probably insoluble within the cell, since electron microscopy reveals the presence of 'inclusion bodies'. In a cell-free extract the fusion protein is also insoluble, but can be solubilized with sodium dodecyl sulfate, and cleaved with cyanogen bromide. The PI that is produced contains incorrect disulfide bonds. After sulfitolysis, the product can be easily purified, renatured, and processed

L3 ANSWER 53 OF 64 MEDLINE
ACCESSION NUMBER: 89003160 MEDLINE
DOCUMENT NUMBER: 89003160 PubMed ID: 2458849
TITLE: The secretion and post translational modification of interferons from Saccharomyces cerevisiae.
AUTHOR: Piggott J R; Watson M E; Doel S M; Goodey A R; Carter B L
CORPORATE SOURCE: G.D. Searle & Co., Limited, Buckinghamshire, UK.
SOURCE: CURRENT GENETICS, (1987) 12 (8) 561-7.
Journal code: 8004904. ISSN: 0172-8083.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19960129
Entered Medline: 19881123

AB Studies with three interferon molecules, IFN-alpha 2, IFN-beta 1, and a "**hybrid**" interferon, IFNX-430 are described which illustrate that both the expression and secretion characteristics of **heterologous** proteins in **yeast** cells reflect properties of the proteins themselves. Recombinant DNA techniques have also been used to demonstrate that the efficient processing of mature **heterologous** proteins from the **yeast** alpha factor secretion leader can be affected by sequences on the carboxyl side of the initial cleavage site. Secretion studies with **heterologous** proteins in S. cerevisiae are aimed at maximising yield, the percentage of extracellular product and correct amino terminus sequence. The results presented here show that all three factors are susceptible to currently unpredictable properties of the foreign sequence. This situation, in turn, means that **heterologous** proteins can be used as tools in the biochemical dissection of the **yeast** secretion process.

L3 · ANSWER 46 OF 64 MEDLINE
ACCESSION NUMBER: 91316134 MEDLINE
DOCUMENT NUMBER: 91316134 PubMed ID: 1859838
TITLE: The use of genetic engineering to obtain efficient production of porcine pancreatic phospholipase A2 by Saccharomyces cerevisiae.
AUTHOR: Bekkers A C; Franken P A; Van den Bergh C J; Verbakel J M; Verheij H M; De Haas G H
CORPORATE SOURCE: Department of Enzymology and Protein Engineering, State University of Utrecht, The Netherlands.
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1991 Jul 23) 1089 (3) 345-51.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199109
ENTRY DATE: Entered STN: 19910922
Last Updated on STN: 19910922
Entered Medline: 19910904

AB We have developed an efficient production system for porcine pancreatic phospholipase A2 in Saccharomyces cerevisiae (baker's **yeast**). The cDNA encoding the prophospholipase A2 was expressed under the control of the galactose inducible GAL7 promotor, and secretion was directed by the secretion signals of **yeast** invertase. This construct yielded up to 6 mg prophospholipase A2 activity per 1 fermentation broth, secreted as a glycosylated invertase prophospholipase A2 **hybrid** protein. Upon genetically deleting the glycosylation site, the level of secretion decreased to 3.6 mg prophospholipase A2 per 1. Changing the invertase secretion signals for an invertase/alpha-mating factor prepro sequence-fusion increased the secretion level up to 8 mg per 1. The secreted non-glycosylated prophospholipase A2 species was correctly processed. Our results demonstrate the promises and limitations for rational design to obtain high level expression and secretion of

L3 ANSWER 14 OF 64 MEDLINE
ACCESSION NUMBER: 1998231060 MEDLINE
DOCUMENT NUMBER: 98231060 PubMed ID: 9569602
TITLE: Expression and secretion of beta-galactosidase in Saccharomyces cerevisiae using the signal sequences of GgpI, the major **yeast** glycosylphosphatidylinositol-containing protein.
AUTHOR: Pignatelli R; Vai M; Alberghina L; Popolo L
CORPORATE SOURCE: Dipartimento di Fisiologia e Biochimica Generali, Universita degli Studi di Milano, Italy.
SOURCE: BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (1998 Apr) 27 (Pt 2) 81-8.
Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980611
Last Updated on STN: 20000303
Entered Medline: 19980529

AB New secretory signals and strategies can be attempted to improve the secretion of **heterologous** proteins of biotechnological interest which encounter difficulties being exported in **yeast**. The GGPI gene of Saccharomyces cerevisiae codes for a 125 kDa glycoprotein transported through the secretory pathway and anchored to the plasma membrane by means of a glycosylphosphatidylinositol. The regions coding for the secretory signal or also for the first 46 amino acids were tested for efficiency in secretion by fusion to the lacZ gene of Escherichia coli resulting in the synthesis of the endoplasmic reticulum-targeted 1-22- and 1-68-GgpIp/beta-gal hybrids. A cytoplasmic form was also examined. The 1-22 beta gal is partially transported to the cell surface and in the medium in an unglycosylated form. The 1-68 beta gal is completely retained in the intracellular membranes and is N-glycosylated in the GgpIp moiety. The amount of **hybrid** protein produced is similar and independent from its targeted site, suggesting that translocation through endoplasmic reticulum is not a limiting step, whereas the amount of active enzyme is from 50 to 80% lower for the endoplasmic reticulum forms compared with the cytoplasmic form. BiP/Kar2p putative precursor is accumulated in cells expressing the endoplasmic reticulum-targeted forms but not in those producing the cytosolic beta-galactosidase or over-expressing an endogenous secretory protein. Thus, glycosylation and abnormal folding rather than over-expression are among the factors responsible for the decreased activity and exit of beta-galactosidase from the endoplasmic reticulum and for induction of BiP. The results obtained indicate that the sole secretory signal of GgpIp is suitable to drive secretion of foreign products with complex folding and point to the importance of the endoplasmic reticulum quality control in the secretion of **heterologous** proteins in **yeast**.

L3 ANSWER 7 OF 64 MEDLINE
ACCESSION NUMBER: 1999421688 MEDLINE
DOCUMENT NUMBER: 99421688 PubMed ID: 10491129
TITLE: Functional complementation analysis of **yeast** bc1 mutants. A study of the mitochondrial import of **heterologous** and **hybrid** proteins.
AUTHOR: van Wilpe S; Boumans H; Lobo-Hajdu G; Grivell L A; Berden J A
CORPORATE SOURCE: Section for Molecular biology, Department of Molcular Cell Biology, BioCentrum, University of Amsterdam, The Netherlands.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 Sep) 264 (3) 825-32.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991014

AB Previous complementation studies with **yeast** bc1 mutants, defective in subunit VII or VIII, using **heterologous** and **hybrid** subunits, suggested that the requirement for import into mitochondria might significantly restrict the scope of this test for compatible proteins. Prediction algorithms indicate that the N-terminal domain of subunit VII contains all known characteristics of a mitochondrial targeting signal, whereas in subunit VIII such a signal is absent from the N-terminal domain, but possibly present in an internal region of the protein. Despite the fact that the characteristics of a mitochondrial import signal are found in the N-terminus of all known subunit-VII orthologues, in vitro import experiments show that the protein of human origin is not imported into **yeast** mitochondria. In vitro import can be restored, however, by replacement of the N-terminal part of the human protein by the N-terminus of the Saccharomyces cerevisiae orthologue, indicating a requirement for species-specific elements. Similar experiments were performed with subunit VIII and orthologues thereof, including a **hybrid** protein in which the N-terminus of the bovine heart orthologue was replaced by that of S. cerevisiae. The ability of **yeast** mitochondria to import this **hybrid** protein, in contrast with the bovine subunit-VIII orthologue itself, indicates that for subunit VIII also the N-terminus, in contradiction of theoretical predictions, contributes to the targeting signal, most likely via species-specific elements. Our findings expose the limitations of the currently available criteria for prediction of the presence and location of a mitochondrial targeting sequence and highlight the necessity of performing separate import studies for interpreting complementation studies as long as the species-specific characteristics of

5 ANSWER 30 OF 33 MEDLINE

ACCESSION NUMBER: 87305591 MEDLINE
DOCUMENT NUMBER: 87305591 PubMed ID: 2957274
TITLE: Detection of **heterologous** fusion proteins in Escherichia coli with a monoclonal antibody.
AUTHOR: Zweig M; Showalter S D; Du Bois G C; Sisk W P; Court D L
CONTRACT NUMBER: NO1-CO-23910 (NCI)
SOURCE: GENE, (1987) 55 (1) 47-53.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19871015

AB Several laboratories have constructed expression vectors for the production of **heterologous** fusion proteins containing the N-terminal 13 amino acids of the bacteriophage lambda cII-coded protein in Escherichia coli. We have prepared a monoclonal antibody to a synthetic peptide having this CII amino acid sequence and have found that this antibody reacts with authentic CII protein in Western blot tests and with most CII peptide-containing fusion proteins in both radioimmunoprecipitation and Western blot assays. However, there are some CII-**hybrid protein** species with which the antibody does not react. Our findings indicate that this antibody is a valuable tool for detecting and purifying expressed proteins and in studying their structure and function.

L5 ANSWER 29 OF 33 MEDLINE
ACCESSION NUMBER: 91144806 MEDLINE
DOCUMENT NUMBER: 91144806 PubMed ID: 2288714
TITLE: Exploiting the cell membrane for the production of **heterologous** proteins in Escherichia coli.
AUTHOR: Lundell D; Lunn C; Greenberg R; Fossetta J; Narula S; Kastelein R; Van Kimmenade A
CORPORATE SOURCE: Biotechnology-Molecular Biology, Schering-Plough Research, Bloomfield, New Jersey 07003.
SOURCE: BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (1990 Oct) 12 (5) 567-78.
Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199104
ENTRY DATE: Entered STN: 19910419
Last Updated on STN: 19910419
Entered Medline: 19910404

AB The bacterial membrane serves both as a cell organelle and as a barrier for segregating the metabolically active cytoplasm from the extracellular milieu. Thus we can use plasmid vectors designed to produce a **hybrid protein** containing an efficient signal peptide coupled to the amino terminus of the cloned **heterologous** protein (secretion cloning vectors) for the production of proteins which are insoluble, proteolytically sensitive, or bacteriocidal when produced in the cytoplasm of Escherichia coli. We demonstrate that human granulocyte-macrophage colony stimulating factor can be isolated as an active species only after transport into the bacterial periplasm. Production of the protein in the bacterial cytoplasm is bacteriocidal. We also demonstrate that biologically active human interleukin 4 appears only after transport of the protein into the bacterial growth medium. The protein forms membrane-associated aggregates in the cytoplasm, and demonstrates an active but nonnative conformation when expressed in the periplasm. This may correlate with the affinity of the interleukin 4 molecule for negatively charged macromolecules, including bacterial membrane components and bacterial lipopolysaccharides, which may alter the